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Scientific Article

Identification of ssr markers associated with resistance to *Liriomyza sativae* in *Cucumis melo*

Identificação de marcadores ssr ligados à resistência a *Liriomyza sativae* em *Cucumis melo*

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ABSTRACT - Melon is an economically important crop worldwide, but pest infestations limit fruit yield and quality. The leafminer fly (*Liriomyza* spp.) is a major pest of melon crops. Therefore, resistant genotypes carrying specific alleles are highly desirable. This study aimed to identify SSR markers associated with melon resistance to the leafminer fly. Crosses were performed between resistant (Rutênio) and susceptible (Vedrantais) plants, generating F_1, F_2, and backcross populations that were evaluated for resistance. Molecular markers were used for genotyping, and segregation tests based on chi -square analyses were conducted to assess genetic distortions. Regression analyses were used to estimate marker effects on phenotypic variation. Among the 192 F_2 plants evaluated, phenotypic segregation followed a 3:1 ratio, consistent with monogenic dominant inheritance. Of the 100 SSR markers tested, 19 were polymorphic, and 13 were selected because they showed no significant segregation distortions. Three markers (CMTA166, CMCT170B, and CMTA134A) explained 5.73%, 4.46%, and 3.32% of the phenotypic variation, respectively. Multiple regression analysis indicated that CMTA166 and CMCT170B jointly explained 9.42% of the variation. Although the identified SSR markers accounted for a relatively small proportion of the phenotypic variation, CMTA166 and CMCT170B showed the strongest association with resistance to *Liriomyza sativae* and may contribute to future marker-assisted selection studies in melon breeding programs.

Keywords: Genetic variability. Leafminer fly. Melon. Microsatellites.

RESUMO - O melão é uma cultura economicamente importante em todo o mundo, mas a incidência de pragas limita a produtividade e a qualidade dos frutos. A mosca-minadora (*Liriomyza* spp.) é uma das principais pragas que afetam o melão. Por esse motivo, genótipos resistentes portadores de alelos específicos são estudados. Este estudo teve como objetivo identificar marcadores SSR associados à resistência do melão à mosca-minadora. Cruzamentos foram realizados entre plantas resistentes (Rutênio) e suscetíveis (Vedrantais), gerando populações F1, F2 e RC, avaliadas quanto à resistência. Marcadores moleculares foram utilizados para genotipagem, e testes de segregação com análises de qui-quadrado avaliaram as distorções genéticas. Análises de regressão estimaram os efeitos dos marcadores na variação fenotípica. Entre 192 plantas F2, a segregação fenotípica seguiu uma proporção de 3:1, consistente com o controle dominante monogênico. Dos 100 marcadores SSR testados, 19 foram polimórficos e 13 foram selecionados por não apresentarem distorções significativas. Três marcadores (CMTA166, CMCT170B, CMTA134A) explicaram 5,73%, 4,46% e 3,32% da variação fenotípica, respectivamente. A regressão múltipla indicou que CMTA166 e CMCT170B, juntos, explicaram 9,42%. Embora os marcadores SSR identificados expliquem uma baixa proporção da variação fenotípica, CMTA166 e CMCT170B apresentaram a associação mais forte com a resistência a *Liriomyza sativae* e podem contribuir para futuros estudos de seleção assistida por marcadores em programas de melhoramento de melão.

Palavras-chave: Variabilidade genética. Mosca-minadora. Melão. Microssatélites.

Conflict of interest: The authors declare no conflict of interest related to the publication of this manuscript.

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INTRODUCTION

Melons (*Cucumis melo* L.) belong to the Cucurbitaceae family and are highly valued worldwide, playing an important role in the global economy. In 2024, melon production in Brazil reached approximately 125,100 tons. Despite fluctuations in production over recent years, melon remains one of the most economically important horticultural crops in the country (IBGE, 2025). Although global production is high, inadequate crop management practices, such as excessive insecticide use and monoculture systems, favor pest outbreaks and increase production losses. In the early 2000s, the leafminer (*Liriomyza* spp.) evolved from a secondary pest into one of major economic importance in melon production (HAMZA et al., 2023), becoming an important limiting factor for crop productivity over time (SILVA JR et al., 2020; LEMOS et al., 2021).

The leafminer fly is a dipteran insect belonging to the Agromyzidae family and is of global importance because it attacks a wide range of crops cultivated worldwide. A molecular identification study revealed that leafminer populations occurring in the Northeast and Southeast regions of Brazil belong to the species *Liriomyza sativae* (FERREIRA et al., 2017; CELIN et al., 2017). The larvae are

responsible for the most severe agronomic damage, penetrating the leaf mesophyll and forming mines that drastically reduce fruit yield and quality due to reductions in photosynthetic activity (COSTA et al., 2017; SADIQ; AL-NADAWI; MAHMOOD, 2020).

The most effective strategy for preventing losses caused by leafminers is the identification of resistance sources for incorporation into breeding programs (OLIVEIRA et al., 2017). These sources are obtained through the inheritance of traits that negatively affect pest development, reducing or preventing insect attack on the plant (OLIVEIRA et al., 2025). This resistance may be classified as antibiosis, when the plant releases compounds that adversely affect the insect, or antixenosis, when plant traits alter insect behavior (DEMIS, 2024).

The identification of resistance sources can be facilitated through the use of molecular markers to detect Quantitative Trait Loci (QTLs) associated with resistance, which are important tools in breeding programs (LEITÃO, 2018). Molecular markers are specific DNA sequences linked to loci of interest and can be used to differentiate individuals based on polymorphisms present in their genomes (MARWAL; GAUR, 2020). Several types of markers are available for genetic studies, including microsatellites or SSRs (Simple Sequence Repeats), ISSRs (Inter Simple Sequence Repeats), and SNPs (Single Nucleotide Polymorphisms), among others. Because each marker type provides different genetic information, selecting the most appropriate marker depends on the goals of the study (AMITEYE, 2021).

Markers associated with resistance QTLs can be used in breeding programs through marker-assisted selection (MAS). However, a major challenge for breeders is identifying QTLs that explain a substantial proportion of the phenotypic variation and are therefore suitable for MAS applications (COBB; BISWAS; PLATTEN, 2019; SAHARAN; MEHTA; MEENA, 2022). QTLs are valuable tools for understanding host pest interactions and identifying resistance mechanisms (HUDSON; BRAWNER, 2025). These chromosomal regions can be associated with genes of interest through the integration of phenotypic and molecular marker data across the genome (CHESNOKOV; KOSOLAPOV; SAVCHENKO, 2020). Therefore, this study aimed to identify SSR markers and genomic regions associated with resistance to *L. sativae*.

MATERIALS AND METHODS

Preparation and Seed Production of the F_2 Generation

The experiment was conducted in a greenhouse at the Federal Rural University of the Semi-Arid Region (UFERSA). Two contrasting melon genotypes previously characterized for their response to *L. sativae* Blanchard (Diptera: Agromyzidae) were used as parental lines: the commercial hybrid Rutênio (yellow melon type), resistant to the pest, and the accession Vedrantais, susceptible to infestation (COSTA et al., 2017).

Controlled hybridizations were performed manually by emasculating female flowers before anthesis and transferring pollen from the male parent using a fine brush. Pollinated flowers were protected with paper bags to prevent unwanted pollination. Because Rutênio is a commercial hybrid and behaves as a heterozygous genotype for resistance (Aa), crosses between Rutênio (Aa) and the susceptible accession Vedrantais (aa) generated an F_1 population expected to segregate at a 1:1 ratio for the resistance trait.

The F_1 plants were evaluated using the same infestation and phenotypic assessment procedures adopted for the F_2 population, and individuals were classified as resistant or susceptible according to larval development and pupation capacity. After phenotypic evaluation, only resistant F_1 individuals, presumed to be heterozygous (Aa), were selected and self-pollinated to produce fruits and seeds, generating the F_2 population. Consequently, the F_2 generation was expected to segregate according to the Mendelian ratio of three resistant plants to one susceptible plant (3:1). Additionally, backcross populations were obtained by crossing F_1 individuals with both the resistant and susceptible parental lines.

Seeds of the parental genotypes were pre-germinated in Petri dishes under controlled temperature (37 °C) and photoperiod (12 h) conditions for 24 h. Temperature, relative humidity, and photoperiod were monitored and maintained using an automated environmental control system installed in the greenhouse. After radicle emergence, seedlings were transplanted into 10-L pots containing a mixture of soil and the commercial substrate Plantmax® at a 3:1 ratio (v/v) and irrigated twice daily. To prevent experimental losses caused by microorganism infestations, the soil was previously autoclaved at 120 °C for 1 h.

Preparation of F_2 Generation Seedlings

Seeds from the generated populations and the parental lines were germinated in 200-cell trays filled with coconut fiber substrate. After the emergence of the first true leaf, seedlings were transplanted into 300-mL pots containing a mixture of coconut fiber and organic compost at a ratio of 3:1 (v/v). The plants were maintained under greenhouse conditions until the leafminer infestation stage.

Infestation with *Liriomyza sativae* under controlled conditions

When plants reached the three-true-leaf stage, approximately 25 days after emergence, they were transferred to the Applied Entomology Laboratory at UFERSA. Infestations were performed according to the methodology described by Ferreira et al. (2022). The plants were placed in cages covered with aphid-proof mesh (50 × 50 × 50 cm), each containing approximately 100 adult leafminer flies from the laboratory colony. The F_2 population, together with the backcross populations, F_1 generation, and parental lines, was infested for 1 h at a density of ten plants per cage. During the infestation period, a feeder containing a 10% honey solution was provided as a food source for the insects. After infestation, the plants were returned to the greenhouse and maintained for 48 h before evaluation.

Evaluation of larval and pupal mortality of *Liriomyza sativae*

Larval and pupal mortality of *L. sativae* was evaluated according to the methodology proposed by Costa et al. (2016). Plants were transported between the greenhouse and laboratory during the evaluation period. Visual assessments were performed on the first two leaves of each plant at 48, 72, and 96 h after infestation. During each assessment, dead larvae were counted, and larval mortality (%) was calculated as the number of dead larvae divided by the total number of observed larvae and multiplied by 100.

After larval development was completed, plants were placed horizontally in Styrofoam trays under controlled temperature (25 ± 5 °C) and relative humidity (60 ± 10%) conditions to allow surviving larvae to exit the leaves and pupate. After five days, pupae were collected, identified, and maintained for 14 days to evaluate adult emergence and estimate pupal mortality. Pupal mortality (%) was calculated by subtracting the number of adults emerging from the total number of pupae, dividing the result by the total number of pupae, and multiplying by 100.

Phenotypic evaluation of resistance in the F_2 population

Following controlled infestation, plants from the F_2 population were phenotypically evaluated for resistance and classified as resistant or susceptible according to the criteria described by Ferreira et al. (2022). Plants were classified as resistant when larvae failed to complete development and to reach pupal stage, whereas plants were considered susceptible when larvae developed normally and successfully pupated.

Phenotypic segregation was analyzed using the chi-square (χ^2) test implemented in PAST version 3.26, comparing observed and expected frequencies according to Mendelian segregation ratios.

Molecular analysis

DNA from the parental genotypes, the F_1 individual, backcross populations, and all F_2 individuals was extracted according to the protocol described by Doyle and Doyle (1987). DNA quantification was performed on 1% agarose gels by comparing sample bands with a molecular weight standard of known concentration. DNA samples were standardized to approximately 10 ng/µL for PCR amplification.

Backcross confirmation was performed through SSR band pattern analysis by comparing the allelic profiles of the progenies with those of the parental genotypes. Individuals exhibiting the expected allelic combination from the recurrent parent and the F_1 genotype were considered confirmed backcrosses.

The molecular analysis began with the amplification of 100 microsatellite markers (Table 1) in the F_1 generation to identify segregating markers. The selected markers were subsequently used to genotype all individuals of the F_2 population. PCR reactions were performed in a final volume of 12 µL containing 10 ng of genomic DNA, 5 µM of each forward and reverse primer, 2 mM of each dNTP, 1× reaction buffer (50 mM KCl and 200 mM Tris-HCl, pH 8.4), 1.5 mM $MgCl_2$, 5 µg BSA, and 1 U Taq DNA polymerase.

Table 1. SSR markers used for the initial screening of polymorphic loci associated with resistance to *Liriomyza sativae* in melon (*Cucumis melo* L.).

SSR Markers				
CMBR2	CMBR143	CMCTN65	CMMS14-1	CMTC51
CMBR3	CMBR145	CMCT134B	CMMS23-13	CMTCN56
CMBR8	CMATN22	CMCTT144	CMMS27-1	CMTCN62
CMBR22	CMAGN33	CMCT160A+B	CMMS31-3	CMTCN 66
CMBR24	CMAGN52	CMGAN12	CMMS34-6	CMTCN67
CMBR25	CMAGN61	CMGAN21	CMMS35	CMTC123
CMBR35	CMAGN68	CMGAN24	CMMS35-1	CMCT126
CMBR64	CMAGN73	CMGAN25	CMMS35-3	CMTC168
CMBR75	CMAGN75	CMGN45	CMMS36-2	CSWCT1
CMBR83	CMAGN79	CMGTA71	CMTCN1	CSWCT02B
CMBR95	CMAT141	CMGAN80	CMTAAT 100	CSWTA05
CMBR96	CMCTN2	CMGAN92	CMTA134A	CSWCT10
CMBR98	CMCTN4	CMGA 104	CMTAA166	CSWCT16B
CMBR105	CMCTN5	CMGA127	CMTA170A	CSWCT29
CMBR108	CMCTN7	CMMS1-7	CMTA170B	CSWCT30
CMBR111	CMTCN8	CMMS2-2	CMTC13	CSWCT33
CMBR120	CMCTN19	CMMS3-1	CMTCN30	Caps P. de I
CMBR125	CMCTN35	CMMS004	CMTCN41	TJ2
CMBR139	CMCTN38	CMMS12-3	CMTC47	SSR02733
CMBR140	CMCTN53	CMMS12-4	CMTCN50	SSR02734

Microsatellite marker amplification consisted of an initial denaturation step at 96 °C for 1 min, followed by 40 cycles of denaturation at 94 °C for 1 min, annealing for 1 min at the specific temperature established for each marker, and extension at 72 °C for 2 min, with a final extension step at 72 °C for 5 min. The annealing temperature of each marker was previously optimized to determine the most suitable amplification conditions for this study.

PCR products were separated on 3% agarose gels stained with ethidium bromide (0.5 µg/mL) in 1× TBE buffer at 120 V for 2–3 h and subsequently visualized under UV light using a gel documentation system (ProteinSimple). Amplified fragment sizes were estimated by comparison with a 100-bp DNA Ladder Plus molecular weight marker (Cellco).

The segregating markers were used to evaluate genotypic frequencies by comparing DNA fragments from the resistant parent (A1) and the susceptible parent (A2), where A1 corresponds to the allele derived from Rutênio and A2 corresponds to the allele derived from Vedrantais. After constructing the polymorphic marker matrix, the chi-square (χ^2) test was used to detect segregation distortion, and false discovery rate (FDR) control was applied according to the method proposed by Benjamini and Hochberg (1995).

Allelic frequencies were estimated from the observed genotypic frequencies. These frequencies were then evaluated under Hardy–Weinberg equilibrium to assess segregation behavior within the population over generations. All analyses were performed using PAST version 3.26.

Linear regression analyses were performed using molecular marker and resistance data. Associations between SSR markers and the resistance locus were evaluated through simple and multiple linear regression analyses, whereas those between QTLs and markers through simple and multiple linear regression. The proportion of phenotypic variation explained by each marker was estimated using the coefficients of determination (R^2 and adjusted R^2). Statistical analyses were performed using SAS software (SAS INSTITUTE, 1999).

RESULTS AND DISCUSSION

Resistance was manifested after interruption of leafminer larval feeding on melon leaves (Figure 1). Our results were based on detailed observations of larval development after infestation, revealing cessation of feeding activity and, consequently, interruption of mine formation (CELIN et al., 2017). Antibiosis resistance is characterized by adverse effects on insect biology, including impairments in development that may be either sublethal or lethal (OLIVEIRA et al., 2022).

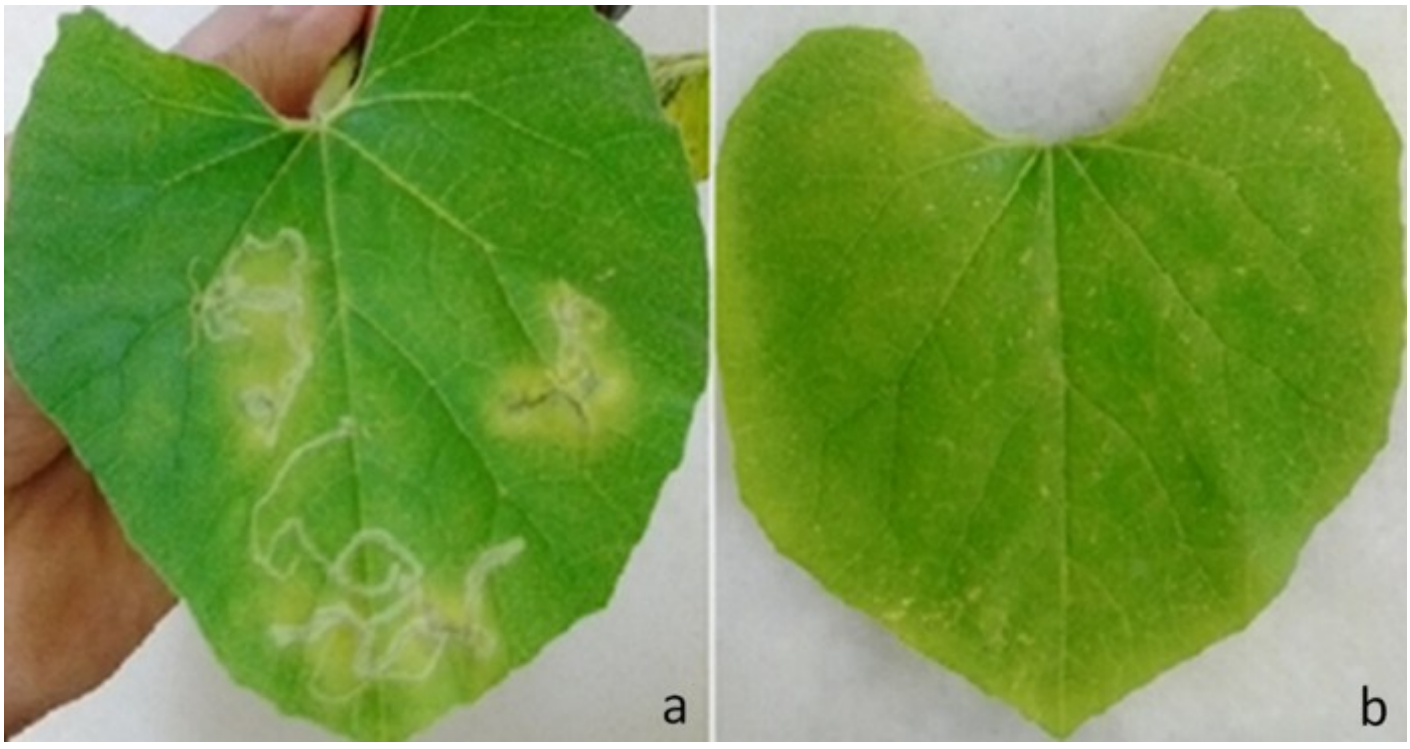


Figure 1. Damage caused by *Liriomyza sativae* larvae on leaves of F_2 melon plants. (a) Susceptible plant. (b) Resistant plant.

Larval mortality revealed a clear contrast between the parental genotypes, with 100% larval mortality in the resistant parent and no mortality in the susceptible parent. These characteristics were maintained in the subsequent generations (F_1, F_2, and backcross populations), confirming the segregation of the resistance trait. All crosses exhibited the expected Mendelian segregation patterns (Table 2).

The parental genotypes showed resistance-to-susceptibility proportions of 1:0 (Rutênio) and 0:1 (Vedrantais), respectively. The F_1 generation segregated in the expected 1:1 ratio of resistant and susceptible individuals, reflecting the heterozygous nature of the Rutênio hybrid. Only resistant F_1 individuals were selected for subsequent crosses. The backcross populations exhibited segregation ratios of 1:1

(F_1 × susceptible parent) and 3:1 (F_1 × resistant parent). In the F_2 population, the resistance trait followed the expected Mendelian segregation ratio of three resistant plants to one susceptible plant.

Our findings corroborate monogenic dominant control of resistance to *Liriomyza sativae* previously reported in melon, in which complete dominance was observed (CELIN et al., 2017). Similar inheritance patterns have been reported in yellow melon genotypes, where resistance to leafminer was controlled by a single dominant gene (FERREIRA et al., 2022). Complete dominance has also been reported in the Nantais Oblong melon lineage, which exhibits antibiosis-mediated resistance against *Liriomyza trifolii* larvae (DOGIMONT, 2011). In contrast, other studies on resistance to *L. sativae* have reported recessive inheritance and incomplete dominance. Understanding the inheritance pattern of resistance is essential for selecting the most appropriate breeding strategy to incorporate this trait into commercial cultivars (OLIVEIRA et al., 2022).

Table 2. Phenotypic segregation of resistance to *Liriomyza sativae* in melon (*Cucumis melo* L.) populations derived from crosses between the Rutênio and Vedrantais genotypes.

Population	Nº of plants		Observed proportion	Expected proportion	χ^2	*P*
	R	S	R:S	R:S		
Rutênio	25	0	1:0	1:0	-------	-------
Vedrantais	0	25	0:1	0:1	-------	-------
F_1	14	11	1.1:0.9	1:1	0.36	0.548
F_2	141	51	2.9:1.1	3:1	0.25	0.617
BCr	18	7	2.9:1.1	3:1	0.12	0.729
BCs	13	12	1:1	1:1	0.04	0.841

Rutênio, resistant parent; Vedrantais, susceptible parent; F_1, first filial generation; F_2, second filial generation; BCr, backcross between Rutênio and F_1; BCs, backcross between Vedrantais and F_1. R, phenotypically resistant individuals; S, phenotypically susceptible individuals; χ^2, chi-square statistic.

Among the 100 microsatellite markers evaluated, 19 (19%) were polymorphic between the contrasting parents for resistance to leafminer (Table 3). Although the polymorphism rate was relatively low, it is important to consider that melon is recognized as the most genetically diverse species within the genus *Cucumis*. This high level of genetic diversity has been demonstrated in studies using both dominant and codominant molecular markers (HU et al., 2019). The limited number of polymorphic microsatellite markers identified in the present study may be associated with the genetic similarity between the parental genotypes used.

Table 3. Polymorphic SSR markers identified between contrasting melon (*Cucumis melo* L.) genotypes differing in resistance to *Liriomyza sativae.*

Marker Name	Forward sequence (5'-3')	Reverse sequence (5'-3')	A.T. (°C)	F.S. (pb)
CMAGN52	CCACCAACATAACACACAAC	CTCTCACACTGTTGGGAAGA	50	135
CMGAN80	TATATTGATTGCTGGGAAAGG	CTTTTTTGGCTTTATTGGGTC	53	159
CMBR120	CTGGCCCCCTCCTAAACTAA	CAAAAAGCATCAAAATGGTTG	55	167
CMBR25	TGGGGTTGTCAATACAGCAA	GGAGTGCGTGGAATGTACG	50	168
CMTCN62	AAGATCGCCTCTATCACAG	ATTTGTACTCCCAACGCATC	50	145
CMAGN79	CTTCACTAAAACTACAAGAG	TTCCAACTTATTCATCCCAC	50	153
CMTAA166	GGAACAGACACCTCTTCTGAG	TCCGTCTACAAGCGTGACTGT	55	167
CMMS004	GCCCAACGGACACACTCACTCACA	GAGGGAGTAAGAATAAGAAGAAGAA	60	190
CMGAN12	TTTTTGTCGTTATATGAGGG	GTTGCATAATGCTAATTTGG	58	179
CMCTN38	TAAAACACTCTCGTGACTCC	GATCTGAGGTTGAAGCAAAG	58	141
CMAGN33	CTGTCTGCTATTCTCCACTTGG	TGTATGCCACGTAGCGAAAC	55	122
CMBR64	ATACAGCAGATCCACAGGGG	ATGGGAGTGTGTGGGATGTA	55	160
CMTA134A	ACGTGCTTCAGTAAACATG	CCCGACATTGAAAACCAACTTC	55	159
CMCT134B	GCTCCTCCTTAACTCTATAC	GCATTATTACCCATGTACGAG	50	125
CMCTT144	CAAAAGGTTTCGATTGGTGGG	AAATGGTGGGGGTTGAATAGG	50	192
CMTA170B	ATTGCCCAACTAAACTAAACC	CACAACACAATATCATCCTTG	55	160
CMTA170A	TTAAATCCCAAAGACATGGCG	AGACGAAGGACGGTTAGCTTT	55	125
SSR2733	TTGTTAGGTAAGCCATGCCC	TTTGCCTGAGGAAGAATCTGA	50	160
CMBR143	ACAAACCGCATCTTCCATTC	TCCAAAAAGGCTAAATCACAAA	53	229

A.T. – annealing temperature in °C; F.S. – fragment size in bp (base pairs). Annealing temperatures were optimized individually for each marker through preliminary testing.

These 19 polymorphic markers were used to genotype all individuals of the F_2 population, allowing the mapping of marker loci and the assessment of their association with resistance to Liriomyza sativae (Figure 2).

The polymorphic markers were used to genotype the 192 individuals of the segregating F_2 population and subsequently evaluate associations between SSR markers and resistance phenotypes. Marker analysis revealed individuals whose allelic profiles were inconsistent with their previously determined resistance phenotypes, suggesting the occurrence of recombination events between the marker loci and the resistance gene.

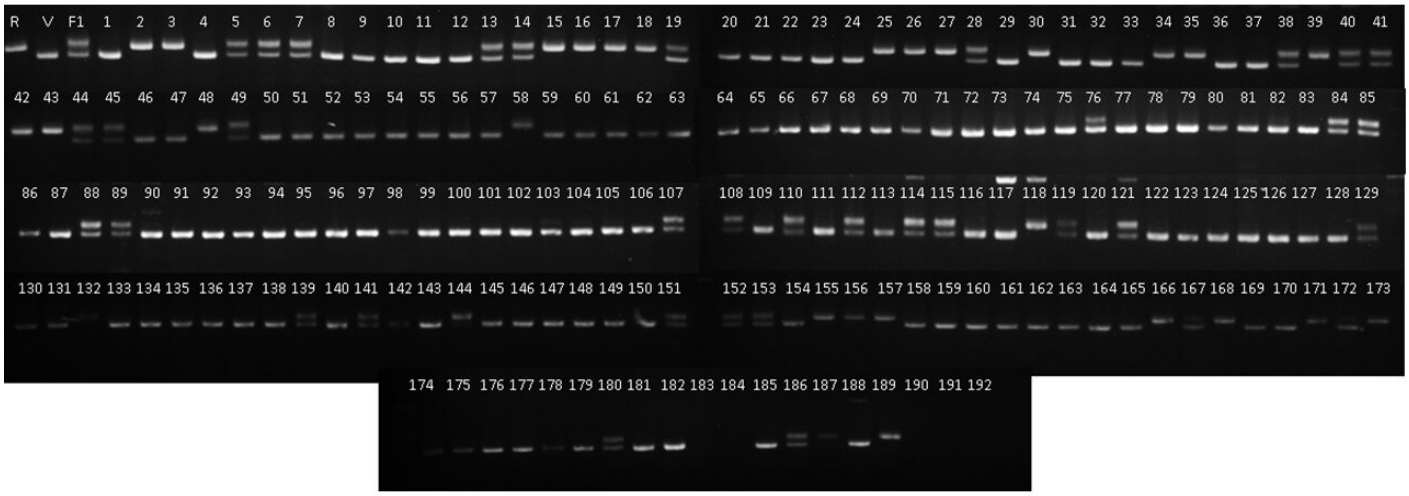


Figure 2. Agarose gel (3%) showing the genotyping profile of 192 F_2 melon individuals derived from crosses between the resistant parent Rutênio and the susceptible parent Vedrantais using the SSR marker CMBR143. R, Rutênio hybrid; V, Vedrantais; F_1, individual obtained from the parental cross.

Discrepancies between marker genotypes and resistance phenotypes may result from crossing over between the marker locus and the resistance locus, reducing linkage between the marker and the resistance allele, and potentially compromising the efficiency of marker-assisted selection (SHARMA; NAIR; CHOUDHARY, 2024).

The application of chi-square (χ^2) tests and false discovery rate (FDR) correction (Table 4) revealed that markers CMBR25, CMTCN62, CMMS004, CMGAN12, CMBR64, and CMBR143 exhibited segregation distortion and deviated significantly from the expected Mendelian segregation ratios. Consequently, these markers were excluded from subsequent QTL analyses. Segregation distortion in codominant markers is a phenomenon frequently reported in several crops, including melon (RITSCHEL et al., 2004).

One possible explanation for the observed discrepancies is distortion at zygotic loci, in which the frequencies of the homozygous genotypes A1A1 and A2A2 deviate from the expected proportions. Furthermore, genotype frequencies for these markers did not conform to Hardy–Weinberg expectations within the F_2 population (MUHAMMADIBROHIM, 2025). Such distortions may reduce the reliability of marker–trait associations and negatively affect the application of these markers in breeding programs.

Nine loci showed a predominance of alleles derived from the resistant parent. In contrast, five loci exhibited a predominance of alleles derived from the susceptible parent. For the remaining four *loci*, no significant differences in allelic frequencies were observed.

Although 13 markers did not exhibit segregation distortion, their number was insufficient to construct linkage groups because marker density was not adequate to generate a map capable of determining the chromosomal positions of these loci. Therefore, the association between the markers and the *locus* underlying resistance to the leafminer fly was evaluated through regression analyses. However, the percentage of phenotypic variation explained by the markers was relatively low (Table 5).

Among the 13 markers without segregation distortion, CMTA166, CMCT170B, and CMTA134A exhibited the highest coefficients of determination (R^2), explaining 5.73%, 4.46%, and 3.32% of the phenotypic variation, respectively. Multiple regression analysis showed that the combination of CMTA166 and CMCT170B explained 9.42% of the observed phenotypic variation. In contrast, markers CMAGN52, CMGAN80, CMBR120, CMAGN79, CMCTN38, CMAGN33, CMCTT144, and CMTA170A showed no significant association with the resistance trait. The remaining markers explained only a small proportion of the phenotypic variation, including CMCT134B (2.78%) and SSR2733 (2.57%).

In studies of melon resistance to downy mildew, microsatellite markers have explained 16.40% and 10.18% of the phenotypic variation, respectively (ALBUQUERQUE et al., 2015). Few studies have reported QTLs associated with resistance to leafminer in melon. For this trait, markers CM321 and CM333 have been reported to explain 30% and 15% of the phenotypic variation, respectively (LEITÃO, 2018). Although resistance in the present study segregated as a monogenic dominant trait, the SSR markers evaluated explained only a small proportion of the phenotypic variation. This finding likely reflects weak linkage between the markers and the major resistance locus rather than evidence of polygenic inheritance. Therefore, the low R^2 values observed should be interpreted as a consequence of the genetic distance between the markers and the resistance locus.

Table 4. Genotypic frequencies, chi-square (χ^2) test results, false discovery rate (FDR) probabilities (%), and allelic frequencies for the F_2 population derived from the cross between the Rutênio and Vedrantais genotypes based on SSR markers.

Marker	Segregation distortion					Allelic frequency	
	Genotypic Frequency						
	A^1A^1	A^1A^2	A^2A^2	χ^2	FDR(%)	f(A^1)	f(A^2)
CMAGN52	57	102	33	6.75	3.42^{ns}	0.56	0.44
CMGAN80	47	99	46	0.19	90.58^{ns}	0.50	0.50
CMBR120	43	105	44	1.69	42.79^{ns}	0.50	0.50
CMBR25	86	77	29	41.37	0*	0.65	0.35
CMTCN62	33	81	78	25.78	0*	0.38	0.62
CMAGN79	28	103	61	12.37	0.27^{ns}	0.41	0.59
CMTAA166	56	111	25	14.69	0.06^{ns}	0.58	0.42
CMMS004	76	100	16	37.8	0*	0.66	0.34
CMGAN12	59	66	67	19.42	0*	0.48	0.52
CMCTN38	67	96	29	15.04	0.04^{ns}	0.60	0.40
CMAGN33	34	115	43	8.36	1.52^{ns}	0.48	0.52
CMBR64	76	87	29	24.7	0*	0.62	0.38
CMTA134A	54	92	46	1	60.65^{ns}	0.52	0.48
CMCT134B	45	101	46	0.53	76.67^{ns}	0.50	0.50
CMCTT144	64	93	35	8.94	1.14^{ns}	0.58	0.42
CMTA170B	57	89	46	2.28	31.96^{ns}	0.53	0.47
CMTA170A	38	88	66	9.5	0.87^{ns}	0.43	0.57
SSR2733	42	108	42	3	22.31^{ns}	0.50	0.50
CMBR143	31	39	122	153.95	0*	0.26	0.74

A1A1, homozygous genotype carrying the allele derived from the resistant parent Rutênio; A1A2, heterozygous genotype carrying alleles derived from both Rutênio and Vedrantais; A2A2, homozygous genotype carrying the allele derived from the susceptible parent Vedrantais; χ^2, chi-square statistic for genotypic frequencies; FDR, false discovery rate. * Significant at the 1% probability level for the expected segregation ratio of 1:2:1; ^{ns}, not significant (markers showing no segregation distortion).

Table 5. Summary of simple and multiple linear regression analyses (Backward method) used to estimate the proportion of phenotypic variation in resistance to *Liriomyza sativae* explained by SSR markers in melon (*Cucumis melo* L.).

Analysis type	Marker	Pr>F	R^2%	R^2aj%
Simple	CMAGN52	0.0647	1.78	1.26
	CMGAN80	0.3832	0.4	0.1
	CMBR120	0.1031	1.39	0.87
	CMAGN79	0.5817	0	0
	CMTA166	0.0008	5.73	5.22
	CMCTN38	0.2222	0.78	0.26
	CMAGN33	0.7207	0	0
	CMTA134A	0.0114	3.32	2.81
	CMCT134B	0.0207	2.78	2.27
	CMCTT144	0.4454	0	0
	CMCT170B	0.0032	4.46	3.96
	CMTA170A	0.2201	0.79	0.26
	SSR2733	0.0261	2.57	2.07
Multiple	CMTA166 + CMCT170B	0.0002	9.42	8.47

Pr>F – probability value associated with the F-test. R^2% - coefficient of determination. R^2aj% - coefficient of determination adjusted.

The results obtained in this study may also reflect the limited ability of the SSR markers employed to detect genomic regions associated with resistance. Albuquerque et al. (2015) reported a polymorphism rate of 29.88% using microsatellite markers, which explained up to 29.52% of the phenotypic variation. Previous studies have demonstrated that even large sets of molecular markers may explain only a limited proportion of the phenotypic variation associated with resistance traits (LEITÃO, 2018). These findings highlight the challenges involved in identifying genomic regions associated with resistance to harmful agents in agricultural crops, such as the leafminer fly, even when a substantial number of markers is evaluated. Consequently, further studies should explore additional classes of molecular markers capable of covering genomic regions not accessed by the SSR markers used in the present study.

Despite the low proportion of phenotypic variation explained, the markers identified in this study may contribute to future investigations aimed at identifying sources of resistance to the leafminer fly. In combination with additional classes of molecular markers, these markers may also support marker-assisted selection and future melon breeding programs.

CONCLUSION

Resistance to *Liriomyza sativae* in the evaluated melon populations exhibited monogenic dominant inheritance. Although the SSR markers explained only a limited proportion of the phenotypic variation, CMTA166 and CMCT170B showed the strongest association with the resistance locus and may contribute to future studies involving marker-assisted selection and the fine mapping of resistance loci in melon.

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